Proc. Natl. Acad. Sci. USA
Vol. 75, No. 1, pp. 351–355, January 1978
Cell Biology

Animal cells reversibly permeable to small molecules

(DNA synthesis/ribonucleotide reductase/hydroxyurea/cytosine arabinosides/baby hamster kidney cells)

JOHN J. CASTELLOT, JR., MICHAEL R. MILLER*, AND ARTHUR B. PARDEE†

Sidney Farber Cancer Institute, 44 Binney Street, Boston, Massachusetts 02115; and Department of Pharmacology, Harvard Medical School, 25 Shattuck Street, Boston, Massachusetts 02115

Contributed by Arthur B. Pardee, October 31, 1977

ABSTRACT **A cell preparation, useful for studying the regulation of metabolism, was developed by making monolayer baby hamster kidney cells permeable. Hypertonically treated cells were permeable to nucleotides, but retained their gross cellular morphology, intact organelles, 100% of their DNA, and 91% of their total protein. The permeable cell synthesized DNA, RNA, and protein rapidly when supplied with the appropriate substrates and cofactors. They either could remain permeable or were able to "reseal" when replaced in complete medium under appropriate conditions. Optimal conditions for DNA synthesis were established for permeable cells, giving rates equal to those of intact cells. Replication rather than repair was shown by the cell-cycle dependence of DNA synthesis and its discontinuous nature. Ribonucleotide reductase was active in permeable cells, permitting equal rates of DNA synthesis when ribonucleotide diphosphates or deoxyribonucleotide triphosphates were provided. Hydroxyurea did not inhibit DNA synthesis in permeable cells supplied with deoxyribonucleotide di- or triphosphates, but drastically inhibited DNA synthesis when ribonucleotide diphosphates were supplied. Hydroxyurea is therefore primarily an inhibitor of ribonucleotide reductase.**

Permeability was reversed, exposing permeable cells to [^{3}H]thymidine triphosphate, which was incorporated, which labeled nuclei of cells that went on to mitosis. The reversible permeability procedure should prove especially useful in studying the functions of poorly penetrating compounds, such as drugs. Intact cells were unaffected by cytosine arabinoside triphosphate, while cells that had been made permeable and resealed were killed.

A major obstacle in attempting to define mechanisms such as those involved in the initiation of S phase and the regulation of DNA synthesis is impermeability of the cell membrane to many molecules, such as nucleotides, cyclic nucleotides, and regulatory proteins. Many studies with intact cells must use intermediary metabolites (e.g., thymidine and uridine), which undergo active transport and multiple enzymatic steps before incorporation into macromolecules. Others involve analogs (e.g., dibutyryl cyclic AMP), which may not precisely mimic the *in vivo* compound.

To circumvent the permeability problem, isolated nuclei and cell lysates have been used (1–6), and have proven useful for studying some aspects of DNA synthesis. Isolated nuclear systems, however, have several drawbacks. (*i*) Isolated nuclei typically carry out DNA synthesis for short periods of time, resulting in replication of only a small fraction of the genome. (*ii*) The rates of DNA synthesis obtained are often substantially lower than the *in vivo* rate. (*iii*) The cytoplasm is stripped away, destroying any structural or spatial nuclear-cytoplasmic interactions. (*iv*) Some of the methods for isolating nuclei use detergents, which severely damage the outer nuclear membrane (7), thus obscuring the role of this structure. (*v*) Complex processes such as mitosis and S phase initiation cannot be studied. It is unclear whether the synthesis observed in isolated nuclei is adequate for investigating many aspects of cell growth.

We have developed two different methods of making animal cells permeable so that exogenous compounds can be introduced into the cells while most macromolecules are retained. This type of cell preparation is analogous to bacteria that have been made permeable (8–10). We report here a simple, reproducible method for making baby hamster kidney (BHK) cells permeable to small molecules that largely overcomes the problems for isolated nuclei. This procedure can be made reversible; these permeable cells "reseal" under appropriate conditions and continue proliferating. We thus report a general method that reversibly alters the permeability of animal cells. A totally different method (using lysolecithin) for reversibly making suspension chinese hamster ovary cells permeable to proteins and small molecules will be published elsewhere (23). The effects of DNA precursors and hydroxyurea on DNA synthesis were investigated in these permeable cells, and ribonucleotide reductase was shown to be active in permeable BHK cells. As an example of the utility of reversible permeability, we have exposed permeable cells to cytosine arabinoside triphosphate (Ara-CTP, a cytotoxic drug excluded by intact cells), resealed them, and demonstrated the inhibition by the drug of cell proliferation.

MATERIALS AND METHODS

[*methyl*-^{3}H]dTTP (10–20 Ci/mmol), [*methyl*-^{3}H]thymidine (35–50 Ci/mmol), L-[4,5-^{3}H(N)]leucine (5 Ci/mmol), and Biofluor scintillation fluid were purchased from New England Nuclear. Nuclease-free pronase was purchased from Calbiochem. All other reagents were obtained from Sigma.

BHK-21/c13 cells were grown in monolayer culture on Falcon flasks and tissue culture dishes in Dulbecco's modified Eagle's medium containing 10% calf serum (Gibco) at 37° in a humidified, 10% CO_2 atmosphere. New cultures of BHK cells were started from frozen stocks at monthly intervals; each new batch of cells was determined to be mycoplasma-free by the method of Schneider *et al.* (11). Cells were synchronized in the G_0 part of the cell cycle by growth in low serum (0.1%) medium for 36–40 hr and released from the G_0 block by adding complete medium (12).

Electron micrographs were kindly prepared and examined by John M. Robinson of the Department of Pathology, Harvard Medical School.

Cells to be made permeable were plated at about 5×10^5 cells per 60-mm tissue culture dish at least 18 hr before an experiment was begun. To make the cells permeable, we washed each plate four times with phosphate-buffered saline and then incubated it at 37° in 2.5 ml of modified Eagle's medium without

Abbreviations: BHK cells, baby hamster kidney cells; Ara-C, cytosine arabinoside; Ara-CTP, cytosine arabinoside triphosphate; Hepes, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid.

* Present address: Department of Biochemistry, West Virginia University School of Medicine, Morgantown, WV 26506.
† To whom reprint requests should be addressed.

serum and containing 4.2 g of NaCl per 100 ml of medium. Permeability was assessed by uptake of Trypan Blue. Generally, 40–50 min was required for about 95% of the cells to stain, at which time the hypertonic medium was removed and a macromolecular synthesis assay solution (described below) was added to the cells.

Permeable cells were resealed in the following manner: Cells were made permeable at a density of 2.0×10^6 cells per 60-mm dish. Test plates were closely monitored for uptake of Trypan Blue during the process. As soon as ≥95% of the cells were stained, the hypertonic medium was aspirated and replaced with warm complete medium. These cells resealed, as shown by loss of staining (see *Results and Discussion*).

Protein synthesis was measured essentially as described by Ochoa and Weinstein (13). At the end of the assay period, cells were washed once with phosphate-buffered saline, and 3 ml of 5% trichloroacetic acid was added for 30 min at 4°. The cells were washed five times with 5% trichloroacetic acid, digested in 1.0 ml of 0.1 M NaOH for 30 min at 37°, and neutralized with 0.1 ml of 1.0 M HCl. The radioactivity of the entire sample was measured in 10 ml of Biofluor in a liquid scintillation counter.

When RNA synthesis was measured after the cells were made permeable, the hypertonic medium was removed and 1 ml of a solution containing 35 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (Hepes) (7.4), 80 mM KCl, 4 mM $MgCl_2$, 7.5 mM potassium phosphate (7.4), 0.75 mM $CaCl_2$, 50 mM sucrose, 0.5 mM $MnCl_2$, 4 mM ATP, 0.25 mM CTP and GTP, 0.01 mM [^{3}H]UTP (4 Ci/mmol), and 200 μg of tRNA was added; the sample was incubated at 37°. At the indicated times, duplicate dishes were removed from the incubator and the assay mixture was aspirated. The cells were washed once with phosphate-buffered saline and processed for liquid scintillation counting as described for protein synthesis.

For DNA synthesis in permeable cells, the hypertonic medium was aspirated, and 1 ml of a solution containing 35 mM Hepes (7.4), 50 mM sucrose, 80 mM KCl, 4 mM $MgCl_2$, 7.5 mM potassium phosphate (7.4), 0.75 mM $CaCl_2$, 10 mM phospho*enol*pyruvate, 1.25 mM ATP, 0.12 mM CTP, UTP, and GTP, and 0.25 mM [^{3}H]dTTP (0.4 Ci/mmol), dATP, dCTP, and dGTP was added. Incubations were at 37°. At the indicated times, duplicate plates were removed from the incubator and processed for scintillation counting as described for protein synthesis.

The DNA of permeable cells incubated in the DNA synthesis mixture was isolated and applied to alkaline sucrose gradients as described by Tseng and Goulian (5). [^{32}P]DNA marker was generously supplied by Michael Kriegler of the Sidney Farber Cancer Institute.

Autoradiography was performed with Kodak NTB-2 nuclear track emulsion according to standard methods.

Protein was determined by the method of Lowry *et al.* (14). DNA was determined by the method of Giles and Mayers (15). Cell number was determined with a Coulter counter.

RESULTS AND DISCUSSION

Characterization of Permeable Cells. A hypertonic modified Eagle's medium made monolayer BHK cells permeable to Trypan Blue while preserving the gross morphology of the cells. The time required for ≥95% of the cells to stain varied from 40 to 50 min; test plates of cells were monitored for Trypan Blue uptake during the permeability procedure to ensure that maximum permeability of the cells was achieved for each experiment.

Electron micrographs of permeable and intact BHK cells were very similar. In particular, the nuclei of permeable cells were indistinguishable from nuclei of intact cells. Both the inner and outer nuclear membranes were intact. The cytoplasm of permeable cells was slightly less dense than in intact cells. The mitochondria appeared intact although slightly swollen. Cytoplasmic features such as filament bundles and endoplasmic reticulum appeared normal in permeable cells.

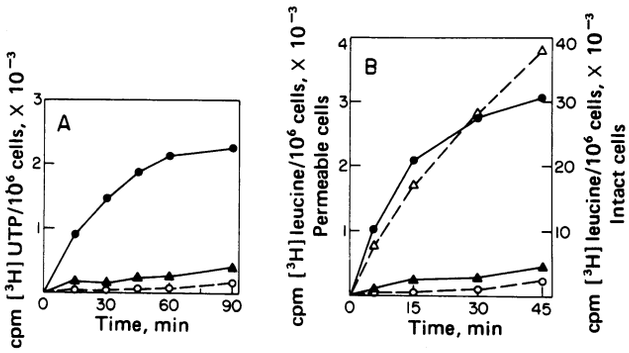

FIG. 1. (*A*) RNA synthesis in permeable BHK cells. Cells were made permeable and their ability to synthesize RNA was assayed. ●—●, Permeable cells, 37°; ▲—▲, permeable cells, plus 3'-deoxyadenosine triphosphate, 37°; O- - -O, permeable cells, 4°. (*B*) Protein synthesis in permeable BHK cells. Cells were made permeable and protein synthesis was assayed by the method of Ochoa and Weinstein (13). ●—●, Permeable cells, 37°; ▲—▲, permeable cells minus ATP, GTP, and phospho*enol*pyruvate, 37°; O- - -O, permeable cells, 4°; △- - -△, intact cells, 37°.

Assays were performed to determine the retention of DNA (15) and protein (14). Both intact and permeable BHK cells contained 65 μg of DNA per 10^7 cells. Intact BHK cells contain 220 μg of protein per 10^6 cells, while after becoming permeable, 10^6 cells contained at least 200 μg of protein, or >91% of the total protein of intact cells.

RNA and Protein Synthesis in Permeable Cells. Hypertonic treatment produced cell preparations with a high degree of physiological integrity that were capable of synthesizing protein, RNA, and DNA. Fig. 1*A* shows that permeable cells incorporated [^{3}H]UTP into acid-insoluble material when supplied with ribonucleotides in the proper reaction mixture. Approximately 95% of the acid-insoluble counts were converted to an acid-soluble form when incubated for 1 hr with 0.1 M NaOH. Permeable cells incorporated [^{3}H]UTP about three times faster than they incorporated [^{3}H]dTTP into DNA (see below). As controls, intact cells did not incorporate [^{3}H]UTP into acid-insoluble material to any significant extent (<5%), and the small amount observed could be eliminated with unlabeled uridine. In agreement with other reports, 3'-deoxyadenosine triphosphate inhibited RNA synthesis 88% in permeable cells but not in intact cells (16).

Fig. 1*B* shows that permeable cells incorporated [^{3}H]leucine into acid-insoluble material. Unlike intact cells, which also incorporated [^{3}H]leucine, protein synthesis in permeable cells was dependent on exogenously supplied ATP and GTP; without ATP and GTP, [^{3}H]leucine incorporation dropped to 15–20% of control (Fig. 1*B*). This requirement demonstrates that permeable cells, rather than the small remaining fraction of impermeable ones, were responsible for the leucine incorporation. While no attempt was made to optimize the protein synthesis mixture, the rate of protein synthesis in permeable cells was approximately 5% of the rate in intact cells (without taking into account internal pools of leucine). Addition of exogenous tRNA did not stimulate incorporation.

DNA Synthesis in Permeable Cells. Optimal conditions for DNA synthesis were determined by varying the concentration

Table 1. Factors affecting DNA synthesis in permeable BHK cells

Additions and omissions	Relative DNA synthesis
Complete	100
$-MgCl_2$	21
+8 mM EDTA	3
$-CaCl_2$	55
+Dithiothreitol	95
−Sucrose	76
−ATP	45
−Phospho*enol*pyruvate	80
−ATP, −phospho*enol*pyruvate	20
+Pyruvate kinase	100
−GTP, −CTP, −UTP	75
+5 mM ATP	55

Complete DNA synthesis solution is described in *Materials and Methods*. Cells were made permeable and exposed to a DNA synthesis solution for 20 min, during which DNA synthesis proceeded linearly (see Fig. 2).

of each assay component over a wide range. The assay solution described in *Materials and Methods* gave optimal rates of DNA synthesis for both short (20 min) and long (90 min) incubations.

DNA synthesis in permeable cells was highly dependent on the concentration of all four dNTPs, with the optimum concentrations at 0.25 mM. DNA synthesis in permeable cells was not significantly reduced (<5%) by addition of 0.1 mM thymidine, demonstrating that synthesis was due to direct incorporation of exogenous nucleotides and not due to degradation of nucleotides to nucleosides before incorporation. Intact cells did not incorporate [^{3}H]dTTP to any significant extent (<5%), and the small amount of DNA synthesis observed was eliminated with unlabeled thymidine. When dATP, dCTP, dGTP, and unlabeled dTTP were omitted from the assay solution, 15% of the control DNA synthesis was observed (Table 1), which may be due to endogenous precursors. Reducing the concentration of any one dNTP also reduced DNA synthesis (Table 2).

Permeable cells supplied with dNDPs as the sole substrates for DNA synthesis synthesized DNA at a 10–15% greater rate than with dNTPs. This effect was seen at optimal (0.25 mM) and half-optimal (0.05 mM) concentrations, and persisted during short or long incubations. Identical results were obtained in Chinese hamster cells made permeable by lysolecithin (23), and suggest that the dNDPs may be the preferred substrates for

Table 2. Effect of nucleotides on DNA synthesis

Additions and omissions	Relative DNA synthesis: Without hydroxyurea	Relative DNA synthesis: With hydroxyurea
0.25 mM dNTPs	100	108
0.05 mM dNTPs	52	
0.25 mM dNDPs	112	119
0.05 mM dNDPs	58	
0.25 mM NDPs	60	
0.50 mM NDPs	98	15
−dCTP	60	
−dCTP, −dATP, −dGTP	15	

The complete DNA synthesis solution is described in *Materials and Methods*. Cells were made permeable and exposed to a DNA synthesis solution for 20 min, during which DNA synthesis proceeded linearly (see Fig. 2).

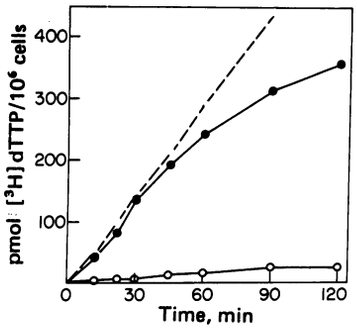

FIG. 2. DNA synthesis in permeable BHK cells. Cells were made permeable and their ability to synthesize DNA was assayed. ●, Permeable cells, 37°; ○, permeable cells, 4°; - - -, calculated rate of DNA synthesis in intact cells (see *Results and Discussion*).

DNA synthesis *in vivo*. Additional studies in permeable cell systems have been undertaken to answer this question.

The presence of 0.1 mM GTP, CTP, and UTP increased DNA synthesis about 25% (Table 1). These ribonucleotides might stimulate DNA synthesis by acting as primers for initiating DNA synthesis (17).

The rate of DNA synthesis in permeable cells was compared to the *in vivo* rate. BHK cells have 65 μg of DNA per 10^7 cells and a doubling time of 16.5 hr (960 min) in our hands. Therefore, an exponential culture of 10^7 BHK cells should synthesize DNA at a rate of 220 pmol of total nucleotides per min. Using data from Fig. 2, permeable BHK cells incorporated total nucleotides at an initial rate of 200 pmol/min per 10^7 cells, or 90% of the calculated *in vivo* rate. This rate dropped to about 30% 2 hr after the cells were made permeable (Fig. 2). Fifteen to twenty percent of the BHK genome was calculated to be replicated during this time.

Permeable cells synthesized DNA at the same rate when supplied with rNDPs as the only precursors as when supplied with dNTPs, indicating that ribonucleotide reductase is present at non-rate-limiting activity (Table 2). We have taken advantage of this activity to provide direct evidence that hydroxyurea primarily inhibits the reductase. Hydroxyurea is a potent inhibitor of this enzyme in cell extracts (18). *In vivo*, hydroxyurea reduces the rate of DNA synthesis and reduces the pool of dGTP in the cells (18–20). These indirect approaches suggest ribonucleotide reductase as the site of action of hydroxyurea on DNA synthesis. *In vivo*, the cell membrane is impermeable to the substrates for the enzyme, which hence depend on prior metabolism. In *in vitro* studies, the enzyme is removed from its physiological setting and from other steps in DNA synthesis, making the physiological relevance of the observed enzyme–inhibitor interaction difficult to assess, especially in light of the greatly decreased activity of the reductase in such systems (18). The permeable cell provides an "*in situ*" approach to the problem; the concentration of substrates and cofactors can be precisely controlled while the enzyme remains in its normal physiological milieu (and likely near its normal cellular activity). When dNTPs or dNDPs were used as DNA substrates in permeable BHK cells, hydroxyurea did not inhibit DNA synthesis (Table 2). However, when the rNDPs were the sole precursors, hydroxyurea reduced DNA synthesis to 15% of control. These results demonstrate a specific inhibition by hydroxyurea on ribonucleotide reduction, since the block can be overcome by adding the products of the reductase (dNDPs). If hydroxyurea had a later site of inhibition, it would have inhibited DNA synthesis in the presence of dNDPs; if its site of inhibition were earlier, it would not have inhibited when rNDPs were added.

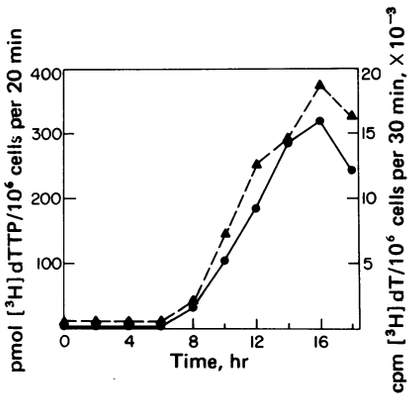

FIG. 3. DNA synthesis in synchronized BHK cells. Cells were arrested in G_0 using low serum (0.1%) medium. Cells were then replaced in fresh medium containing 10% serum. At intervals after release, some cells were made permeable and DNA synthesis was measured using [^{3}H]dTTP (●—●). Other cells were left impermeable and DNA synthesis was assayed by adding 2 μCi of [^{3}H]thymidine per ml of medium for 30 min at 37° (▲- - -▲). Cells were then processed for scintillation counting.

Autoradiography showed that at the cell densities used in a typical experiment, 31% of the intact cells and 26% of the permeable cells were synthesizing DNA. Nearly the same fraction of cells synthesizing DNA *in vivo* synthesized DNA when permeable.

Evidence for Replicative DNA Synthesis in Permeable Cells. The high rate of DNA synthesis observed in permeable cells suggested that we were observing replicative rather than repair synthesis. The replicative nature of the newly made DNA was established by the following line of evidence:

(*i*) The DNA synthesized by permeable cells was cell-cycle dependent. BHK cells were synchronized in G_0 and then released. Then at intervals, some cells were made permeable and [^{3}H]dTTP incorporation was measured. Other cultures were not made permeable and [^{3}H]thymidine incorporation was measured. As Fig. 3 shows, very little incorporation was observed in either intact or permeable G_0 or G_1 cells. When intact cells entering S started to incorporate [^{3}H]thymidine rapidly, permeable cells began incorporating [^{3}H]dTTP at the same time. Cells synchronized in S and then made permeable incorporated 3–4 times as much [^{3}H]dTTP as the same number of exponentially growing cells permeabilized and assayed under the same conditions. Since about one-third of the cells in an exponential culture are in S (see above), the stimulation observed in synchronized cultures is consistent with essentially all the permeable cells in S engaging in scheduled DNA synthesis.

(*ii*) The DNA synthesized by permeable cells was discontinuous, as is DNA synthesis in intact prokaryotes and eukaryotes (17, 21, 22). As shown in Fig. 4, after a 10-sec "pulse", 50% of the DNA was small (4 S). Essentially all this short DNA was chased into large pieces ($\geq 5 \times 10^6$ daltons). This suggests that permeable BHK cells are capable of initiation as well as elongation of Okazaki fragments. Since 15–20% of the genome is replicated in permeable cells (see above), it seems likely that initiation of replicons is occurring as well.

Evidence That the Permeability Is Reversible. As a first test of the reversibility of the procedure, cell number was measured as a function of time after all cells were made permeable and resealed, (see *Materials and Methods*). After a short lag, Trypan Blue-permeable cells resumed the same growth rate as untreated cells (not shown). Since the increase in cell number after cells were made permeable clearly indicated that the treatment left the previously permeable cells viable, the following experiment was done to directly demonstrate the permeability and subsequent capacity of the cells to continue to cycle. Cells were made permeable and then exposed for 2 min to the DNA synthesis solution, omitting unlabeled dTTP. Thymidine (0.1 mM) was present in the assay solution to suppress any incorporation due to [^{3}H]thymidine contaminant in the [^{3}H]dTTP (or produced during the experiment) which might be incorporated by impermeable cells or by permeable cells after they resealed. The DNA synthesis solution was aspirated and replaced with complete modified Eagle's medium twice at 15-min intervals. As controls, intact cells were also exposed to the same [^{3}H]dTTP-containing DNA synthesis solution for 2 min. Other intact cells also were exposed to 5 μCi of [^{3}H]thymidine per ml in complete medium for 10 min, then placed back in fresh medium. At 1-hr intervals after exposure to radioisotope, cells were fixed and prepared for autoradiography. Labeled mitoses were counted (Fig. 5). The peak of percent labeled mitoses in permeable cells occurred at the same time as in the intact cells exposed to [^{3}H]thymidine. Intact cells exposed to [^{3}H]dTTP did not show labeled mitoses at any time. Permeable cells can thus incorporate [^{3}H]dTTP in S phase and then progress through the cell cycle to mitosis, a process complex enough to require the cells to reseal.

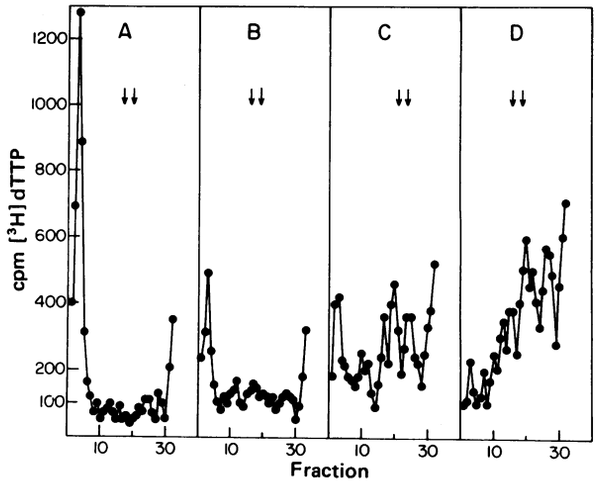

FIG. 4. Discontinuous DNA synthesis in permeable BHK cells. Permeable cells were exposed to the DNA synthesis solution omitting unlabeled dTTP for 10 sec; this solution was aspirated and replaced with the DNA synthesis solution containing 0.25 mM dTTP and no [^{3}H]dTTP for (*A*) 0, (*B*) 1, (*C*) 2, or (*D*) 5 min, after which the reaction was stopped and the DNA was isolated and applied to alkaline sucrose gradients as described (5). Direction of sedimentation is from left to right. Arrows indicate the position of 16S and 18S [^{32}P]DNA marker. Total counts applied to each gradient: (*A*) 8100; (*B*) 8000; (*C*) 10,600; (*D*) 11,800.

In another set of experiments to demonstrate resealing, cells were arrested in G_1 then released and allowed to enter S. These cells were made permeable and exposed to complete modified Eagle's medium containing 20 μM Ara-CTP. This medium was replaced after 15 min with complete medium without Ara-CTP. As controls, intact cells were exposed to Ara-CTP under the same conditions. Other cultures of intact cells were exposed to 20 μM cytosine arabinoside (Ara-C). The viability of drug-treated cells was assayed by trypsinizing and replating the cells and counting colony formation 1 week later. As Table 3 shows, permeable cells treated with Ara-CTP were killed, as were intact cells treated with Ara-C. Intact cells were unaffected by exposure to Ara-CTP; permeable cells were unaffected by exposure to Ara-C. These results demonstrate the utility of this

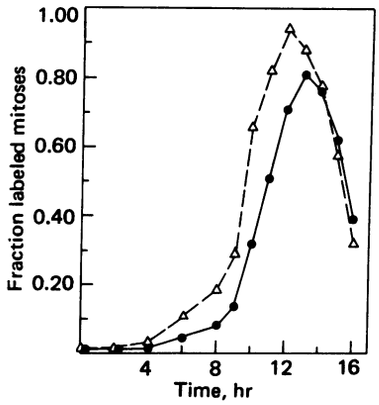

FIG. 5. Labeled mitoses in permeable and resealed BHK cells. Permeable and intact cells were exposed to brief pulses of [^{3}H]dTTP and [^{3}H]thymidine, respectively. The permeable cells were allowed to reseal. At intervals after exposure to radioisotope, the cells were fixed and processed for autoradiography and the fraction of labeled mitoses was determined. To increase the total number of mitoses observed, cells were arrested in G_0, released, and exposed to radioisotope at a time when most of the cells were in early S. (This accounts for the longer than normal G_2 lag before labeled mitoses were observed.) ●—●, Permeable cells; △- - -△, intact cells.

system for directly examining effects of various poorly penetrating compounds. This experiment also demonstrates that becoming permeable and resealing does not affect the viability of BHK cells.

Rationale and Uses for Permeable Cells. This hypertonic procedure for making cells permeable was developed for several reasons. (*i*) A permeable cell overcomes the impermeability of intact cells while still providing a more physiological milieu than isolated nuclei for studying events in which nonpenetrating or actively transported molecules participate; (*ii*) no reversible method for altering the permeability of animal cell membranes existed; (*iii*) no method existed for making monolayer cells permeable; (*iv*) BHK cells can be synchronized and transformed (by viruses), making it possible to examine differences in the regulation of DNA synthesis in normal and transformed variants of the same cell line; and (*v*) we have concurrently developed a method for making suspension Chinese hamster ovary cells permeable. Results obtained in one system can be verified or extended in an independent system.

Table 3. Effect of cytosine analogs on viability of permeable and intact BHK cells

Treatment	Colonies	% control
Intact cells, no drug (control)	95	100
Intact cells with Ara-C	38	40
Intact cells with Ara-CTP	101	106
Permeable, resealed	93	98
Permeable with Ara-C, resealed	107	112
Permeable with Ara-CTP, resealed	49	52

S phase cells were treated as described in *Results and Discussion*. The concentration of both Ara-C and Ara-CTP was 20 μM. Cells were exposed to drug for the same length of time (15 min) in all cases. They were suspended with trypsin and counted, and 500 cells were plated on 100-mm petri dishes. The clones were counted 8 days later after crystal violet staining.

Permeable BHK cells retain a high degree of structural and physiological integrity. In particular, several key enzymes involved in DNA synthesis function at normal physiological activities. We have initiated studies on the allosteric effectors of ribonucleotide reductase, the role of small molecules (nucleotides and cyclic nucleotides) in regulating initiation of S phase, and the role of dNDPs as substrates for DNA synthesis. The reversibility of this treatment is especially useful in that compounds normally excluded from mammalian cells can be directly applied to a permeable cell, the cell can be resealed, and long-range effects studied in a now intact cell.

We acknowledge the technical assistance of Ms. Carmin Karasic. We thank Dr. Rolf Kletzien and Dr. Neil Howell for helpful discussions and critical comments on the manuscript. We also thank David S. Schneider for preparing the manuscript. This work was partially supported by U.S. Public Health Service Predoctoral Traineeship 5T32CA-09031-02 to J.J.C., U.S. Public Health Service Research Fellowship 5F32GM-05349-02 to M.R.M., and U.S. Public Health Service Research Grant CA-18612 to A.B.P.

1. De Pamphilis, M. L. & Berg, P. (1975) *J. Biol. Chem.* **250,** 4348–4354.
2. Friedman, D. L. & Mueller, G. C. (1968) *Biochim. Biophys. Acta* **161,** 455–468.
3. Krokan, H., Cooke, L. & Prydz, A. (1975) *Biochemistry* **14,** 4233–4237.
4. Thompson, L. R. & Mueller, G. C. (1975) *Biochim. Biophys. Acta* **378,** 344–353.
5. Tseng, M. J. & Goulian, M. (1975) *J. Mol. Biol.* **99,** 317–337.
6. Brown, R. L., Clark, R. W., Chu, F.-J. & Stubblefield, E. (1977) *Exp. Cell Res.* **104,** 207–214.
7. Blobel, G. & Potter, V. R. (1966) *Science* **154,** 1662–1665.
8. Buttin, G. & Kornberg, A. (1966) *J. Biol. Chem.* **241,** 5419–5427.
9. Moses, R. E. & Richardson, C. C. (1970) *Proc. Natl. Acad. Sci. USA* **67,** 674–678.
10. Vosberg. H.-P. & Hoffman-Berling, H. (1971) *J. Mol. Biol.* **58,** 739–754.
11. Schneider, E. L., Stanbridge, E. J. & Epstein, C. J. (1974) *Exp. Cell Res.* **84,** 311–318.
12. Hamlin, J. L. & Pardee, A. B. (1976) *Exp. Cell Res.* **100,** 265–275.
13. Ochoa, M., Jr. & Weinstein, I. B. (1964) *J. Biol. Chem.* **239,** 3834–3842.
14. Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193,** 265–275.
15. Giles, K. W. & Mayers, A. (1965) *Nature* **206,** 93.
16. Suhadolnik, R. J. (1970) *Nucleoside Antibiotics* (John Wiley & Sons, New York).
17. Edenberg, H. J. & Huberman, J. A. (1975) *Annu. Rev. Genet.* **9,** 245–284.
18. Reichard, P., Canellakis, Z. N. & Canellakis, E. S. (1961) *J. Biol. Chem.* **236,** 2514–2519.
19. Skoog, L. & Bjursell, G. (1974) *J. Biol. Chem.* **249,** 6434–6438.
20. Walters, R. A., Tobey, R. A. & Ratliff, R. L. (1973) *Biochim. Biophys. Acta* **319,** 336–347.
21. Kornberg, A. (1974) *DNA Synthesis* (Freeman, San Francisco, CA), pp. 188–191.
22. Prescott, D. M. (1976) *Reproduction of Eukaryotic Cells* (Academic Press, New York), pp. 55–59.
23. Miller, M. R., Castellot, J. J. & Pardee, A. B. (1978) *Biochemistry,* in press.